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Brief Report

Relationship Between Toll-Like Receptor 2 Nonsynonymous Single Nucleotide Polymorphisms and the Effectiveness of Bacille Calmette-Guérin Immunotherapy in Preventing Recurrence of Superficial Bladder Cancer: A Prospective Study

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ABSTRACT

BACKGROUND: Intravesical Bacille Calmette-Guérin (BCG) immunotherapy has been used for several decades as a prophylactic approach against recurrence of superficial bladder cancer. However, its effectiveness has been both variable and unpredictable. Typically, cancer BCG-immunotherapy aims to redirect or modulate both innate and adaptive immune responses. The consequences of gene polymorphisms in several key immunoregulatory molecules on the heterogeneity of the response to BCG-immunotherapy have been investigated.

OBJECTIVE: The aim of this study was to evaluate the association of toll-like receptor (TLR) 2 polymorphisms (arginine to glutamine substitution at position 753 [Arg753Gln] and arginine to tryptophan substitution at position 677 [Arg677Trp]) and the outcome of BCG-immunotherapy.

METHODS: This prospective study was conducted during a 3-year period from June 2006 to July 2009. Consecutive patients were recruited during a 1-year period and followed for 2 years at the Department of Urology, Charles Nicolle Hospital, Tunis, Tunisia. Patients with superficial bladder tumors at stage Ta (noninvasive papillary carcinoma) or T1 (where the tumor has grown from the layer of cells lining the bladder into the connective tissue below but has not grown into the muscle layer of the bladder) of any grade were eligible; carcinoma in situ cases were excluded. The TLR2 Arg753Gln and Arg677Trp polymorphisms were studied

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in peripheral blood DNA from patients treated with BCG-immunotherapy after transurethral resection.

RESULTS: A total of 112 consecutive patients were enrolled (101 men and 11 women; mean age, 63.9 years [range, 25–85 years]) and completed the 2-year follow-up. Polymerase chain reaction amplification followed by direct sequencing of the region containing the TLR2 single-nucleotide polymorphism (SNP) of interest did not detect Arg753Gln or Arg677Trp in any of the study participants belonging to either of 2 groups: responders (n = 67) and nonresponders (n = 45) to BCG-immunotherapy.

CONCLUSIONS: No patients included in the study were found to have the 2 known TLR2 nonsynonymous SNPs, and the relative importance of these polymorphisms could not be definitely determined. However, a significant proportion of patients without these polymorphisms responded to BCG-immunotherapy, suggesting that these genetic variants are not critical in the effectiveness of this approach for preventing recurrence of the tumor. (*Curr Ther Res Clin Exp.* 2010;71:398–407) © 2010 Elsevier HS Journals, Inc.

KEY WORDS: genetic polymorphism, BCG-immunotherapy, TLR2, bladder, cancer.

INTRODUCTION

Toll-like receptors (TLRs) are primary transmembrane proteins of immune cells that play a critical role in innate and adaptive immunity. TLRs have been determined as playing a central role in the detection of pathogen-associated molecular patterns and to mediate cellular responses.[1] Currently, 13 TLRs (TLR1–TLR13) have been identified in mammalian species, including 11 in humans.[2] TLR2 has been found to be important in the control of mycobacterial infections.[3] Mycobacterial agonists of TLR2 include lipoproteins and glycolipids.

Intravesical *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) treatment is effective against carcinoma in situ and is used as a prophylaxis against recurrence of superficial bladder cancer.[4] However, its outcome is unpredictable. Understanding the mechanisms that underlie its success and eventually identifying genetic markers that might help predict the response of patients with bladder cancer may be critical to better adapt the present BCG-immunotherapy protocols. Indeed, the association between gene polymorphisms in several key immunoregulatory molecules (eg, natural resistance-associated macrophage protein 1, cytokines and their receptors) and the response to BCG-immunotherapy have been investigated.[5,6] Interestingly, several investigators have found that single-nucleotide polymorphisms (SNPs) in human TLR2, a pattern recognition receptor involved in mycobacteria-mediated intracellular signaling,[7] might increase the risk for mycobacterial infections like tuberculosis[8] and leprosy.[9,10] Indeed, the polymorphism of TLR2 (substitution of an arginine to a tryptophan at position 677 [Arg677Trp]) was found, in initial studies, to be associated with lepromatous leprosy in Korean patients[11] and with tuberculosis in Tunisian patients.[12] Another polymorphism of TLR2 (arginine to glutamine substitution at position 753 [Arg753Gln]) was found to be associated with tuberculosis in Turkish patients.[13]

While the overall consequences of BCG binding to the uroepithelial surface are poorly understood,[4] it is clear that BCG interaction with uroepithelial cells is active rather than passive. BCG adherence induces the expression of genes by bladder tumor cells, such as interleukin (IL)-6 and tumor necrosis factor α,[14] as well as IL-8.[15] Interestingly, BCG adherence stimulates tumor cell expression of IL-6 through an immediate-early pathway requiring the signal transducers nuclear factor kappa-light-chain-enhancer of activated B cells and activator protein 1.[16] This might occur through adhesion-mediated signaling by receptor interaction or through an attachment-facilitated presentation of conserved microbial patterns to TLRs on uroepithelial cells.[15] The present study focused on the detection of nonsynonymous SNPs in the TLR2 coding region and their possible association with BCG immunotherapy response in Tunisian patients with superficial bladder cancer.

PATIENTS AND METHODS

Patients

This prospective study was conducted during a 3-year period from June 2006 to July 2009. Consecutive patients, recruited during a 1-year period, underwent complete transurethral resection (TUR) of the bladder tumor and were followed for 2 years at the Department of Urology, Charles Nicolle Hospital, Tunis, Tunisia. No patients were excluded. Patients were eligible for enrollment after histologic confirmation of superficial bladder cancer and treatment by TUR followed by a complete BCG-immunotherapy instillation protocol. Patients with tumors at stage Ta (confined to the urothelium) or T1 (extending to the lamina propria but superficial to the muscularis propria) of any grade were eligible.[17] Patients were treated by the following adopted protocol: 6 weekly intravesical instillations of BCG (BCG Pasteur strain, 50 mg in 50 mL saline) and then by 12 additional monthly instillations. Patients were followed every 3 months for 24 months; at each follow-up, urine cytology and cystoscopy status were checked. A cystoscopic biopsy was taken from any visible lesion to confirm histology. Patients' follow-up was calculated as the number of months from the date of surgical procedure and diagnosis to the date of the last control cystoscopy. *Responders* to BCG-immunotherapy were defined as patients who did not have evidence of recurrence of their tumor during the 24-month follow-up, while *nonresponders* did have evidence of recurrence. The end point for follow-up was either development of recurrence or the termination date of the study. The statistical analysis was conducted by correlating the potential genetic polymorphisms and recurrence of the tumor in order to identify a predictive recurrence-free survival factor. Written informed consent to participate in the investigation was obtained from all subjects. The Tunisian population is in the Hardy-Weinberg equilibrium. Indeed, both allele and genotype frequencies remain constant as demonstrated through the analysis of 10 short tandem repeat markers validated for use in Tunisian national forensic biology (D. Fathallah, oral communication, April 1998). During initial diagnosis, peripheral blood samples were collected from all patients into EDTA tubes for DNA extraction.

STUDY DESIGN

Genomic DNA was extracted from 5 mL of EDTA-anticoagulated blood using a phenol-chloroform procedure. To determine the TLR2 genotype, the genomic DNA was amplified using forward (5'-GTGTCGGAATGTCACAGGAC-3') and reverse (5'-CCTCAAATGACGGTACATCCA-3') primers that span the region containing the Arg677Trp and Arg753Gln polymorphisms. Polymerase chain reaction (PCR) was performed (Model 9700 Gene Amp PCR system, Applied Biosystems, Foster City, California) in a total volume of 50 µl consisting of 5 µL of 10 µL reaction buffer (20 mM Tris hydrogen chloride [pH 8.8], 50 mM potassium chloride, 15 mM magnesium chloride), 0.5 µL of deoxynucleoside triphosphate mix (25-mM solution), 5 U of DNA polymerase (AmpliTaq, Amersham Biosciences, Little Chalfont, United Kingdom), 25 pmoL of each primer, and 5 µL of genomic DNA (100 ng). PCR was performed under the following conditions: 5 minutes of initial denaturation at 95°C, 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, followed by one elongation step at 72°C for 1 minute. PCR products were subjected to electrophoresis in a 1% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light. All nucleotide sequences were obtained using a cycle sequencing kit (BigDye Terminator Kit, Applied Biosystems, Little Chalfont, United Kingdom) and an automated sequencer (Model 3100, Applied Biosystems, United Kingdom) with 1 µL of the reverse primer (5'-CCTCAAATGACGGTACATCCA-3'). All quality controls for PCR and direct sequencing were rigorously followed. Sequence alignment and SNP search were conducted using BioEdit software (Centre National de Génotypage, Evry, France).

RESULTS

A total of 112 patients were enrolled including 101 male and 11 female with a sex ratio of 9.18 (mean age, 63.9 years [range, 25–85 years]). All patients were Tunisian. All enrolled patients survived to the end of the 24-month follow-up and were included in the genetic study. BCG-immunotherapy was conducted according to the International Protocol for administration. Furthermore, and according to the World Health Organization classification[18] among nonresponders, the tumor grading was established as low malignant potential papillary carcinoma (G1) in 15 patients, intermediate (G2) in 21 patients, and high (G3) in 9 patients; while among responders, 29, 28, and 10 patients were classified as G1, G2, and G3, respectively. The cohort of patients included 67 responders (59.8%) and 45 nonresponders (40.2%) to BGC-immunotherapy.

PCR amplification followed by direct sequencing of the region containing the TLR2 SNPs of interest detected neither C to T mutation at position 2029 that results in Arg677Trp (**Figure 1**), nor G to A mutation at 2251 that results in Arg753Gln (**Figure 2**) in any of the study participants.

DISCUSSION

Two SNPs in the intracellular toll/IL-1 receptor domain of TLR2 have been described that influence cell-type specific activation, nature, and signal transduction pathways of the immune response.[9,19] These polymorphisms, Arg677Trp and Arg753Gln, have

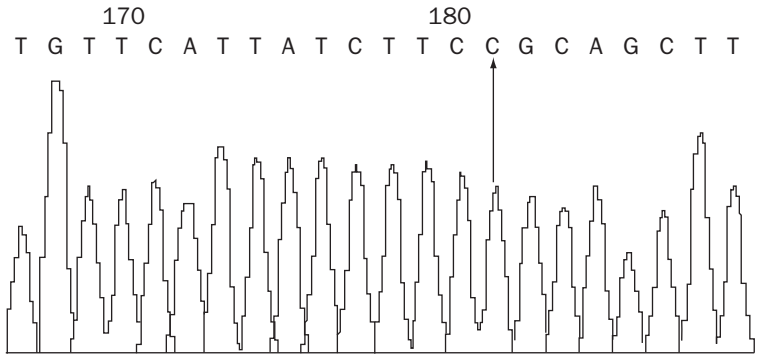

Figure 1. Sequencing results of arginine to tryptophan substitution at position 677 in toll-like receptor 2 genomic DNA. Homozygous C/C (wild type/wild type) genotype.

been reported in some studies to be associated in humans with susceptibility to tuberculosis due to *Mycobacterium tuberculosis*, as well as to susceptibility to leprosy due to *Mycobacterium leprae*.[11–13] In this study, we did investigate the presence of these polymorphisms and their eventual association with the favorable/unfavorable outcome of *M bovis*–BCG-immunotherapy when used as a prophylactic approach against the recurrence of superficial bladder cancer patients. BCG interaction with uroepithelial and, eventually, innate immunity cells is thought to mediate an inflammatory immune response that is beneficial against the recurrence of the disease in a significant score of patients with superficial bladder cancer.[15] TLR2 as a major pattern recognition receptor involved in mycobacterium-mediated intracellular signaling may be critical in this regard and its potential polymorphisms could be predictive factors for the outcome of BCG-immunotherapy.

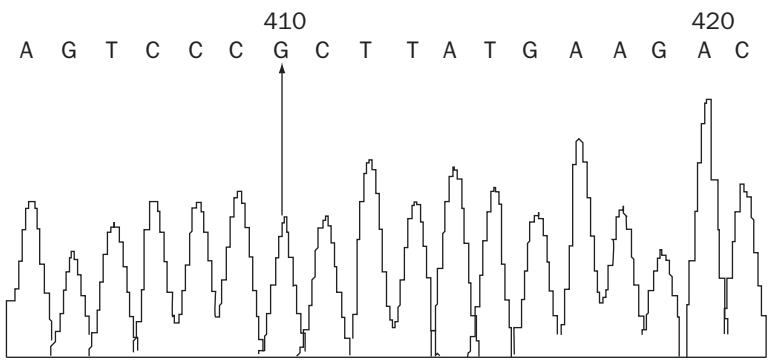

Figure 2. Sequencing results of arginine to glutamine substitution at position 753 in toll-like receptor 2 genomic DNA. Homozygous G/G (wild type/wild type) genotype.

The present study is, to the best of our knowledge, the first to evaluate such a hypothesis. It was designed to recruit all consecutive patients attending a single medical center. All patients approached for enrollment were eligible according to the inclusion criteria. None among them was excluded and all of them survived the 24-month follow-up. The number of patients enrolled was limited to those consecutively identified during a 1-year period. At the end of the 24-month follow-up, they were subdivided into 2 groups of responders/nonresponders to BCG-immunotherapy and recruited for genetic study.

The present results found that the SNP Arg677Trp, which was expected to give rise to an anergic phenotype for mycobacterial infections, was not found in these Tunisian patients, regardless of their BCG immunotherapy response status. Indeed, 224 alleles in this study were observed and showed no evidence for the presence of this TLR2 SNP. Interestingly, this is consistent at least in regard to Arg677Trp with the results of recent studies that identified no individuals carrying this polymorphism in populations including Indians,[20] Koreans,[11] whites,[21,22] and Chinese.[11] Indeed, Malhotra et al[20] revisited the association between this polymorphism and leprosy, and demonstrated that the TLR2 Arg677Trp is not a real polymorphism. They found that this variant is present in the duplicated region of exon 3 situated ~23 kb upstream of the TLR2 gene with 93% homology with the authentic exon 3 of TLR2. In consequence, the use of primers designed to exclusively amplify the TLR2 authentic sequence is critical to avoid any misinterpretation of the results. In the present study, we used such primers in contrast to the first study of tuberculosis in Tunisian patients where primers also amplifying the pseudogene had been used.[12]

The Arg753Gln SNP has been found to be present in 9% to 10% of whites including German,[23] Brazilian,[24] Italian,[25] and Belgian populations[26] (**Table**), and has been found to be associated with tuberculosis, asthma, and septic shock.[13,20,27] Mutlubas et al[28] found an association between chronic allograft nephropathy and this polymorphism. In our study, 224 alleles in total have been studied and showed no evidence for the presence of this TLR2 SNP. Along with our study, other studies have found that this polymorphism was absent among Korean and Chinese populations.[29–31]

This study did have important limitations. No patients with the polymorphism were identified, making it impossible to evaluate the relative importance of the polymorphism for BCG efficacy. However, the fact that there were responders and nonresponders in the cohort suggests that the polymorphism is not critical for BCG efficacy. Further studies using larger cohorts are needed to better assess the role of these polymorphisms, if any.

Although BCG-immunotherapy after initial TUR in patients with superficial bladder cancer is more effective than chemotherapy, some patients are at greater risk for recurrence and progression than others. Genetic factors may contribute to this heterogeneous and, to date, unpredictable response.

CONCLUSIONS

In this prospective study, none of these Tunisian patients with superficial bladder cancer were found to have either of the 2 known TLR2 nonsynonymous SNPs and the

Table. Genotype frequencies for variants in the toll-like receptor 2 (TLR2) gene. Data are number (%) of patients.

Study (Year)	Disease Group	Genotype Frequency			Ethnic Group
		CC	CT	TT	
Arg677Trp					
Ryu et al (2006)[31]	Nontuberculosis lung disease	80 (100.0)	0	0	Korean
Ben-Ali et al (2004)[12]	Tuberculosis	2 (6.1)	31 (93.9)	0	Tunisian
	Control	23 (69.7)	10 (30.3)	0	
Kang and Chae (2001)[11]	Lepromatous leprosy	35 (28.9)	41 (33.9)	45 (37.2)	Korean
	Tuberculoid leprosy	10 (100.0)	0	0	
	Control	0	0	0	
Present study	Superficial bladder cancer	112 (100.0)	0	0	Tunisian
Arg753Gln		GG	AG	AA	
Ryu et al (2006)[31]	Nontuberculosis lung disease	80 (100.0)	0	0	Korean
Ogus et al (2004)[13]	Tuberculosis	124 (82.1)	13 (8.6)	14 (9.3)	Turkish
	Control	107 (92.2)	7 (6.0)	2 (1.7)	
Moens et al (2007)[26]	Pneumococcal disease	93 (93.9)	6 (6.1)	0	Belgian
	Control	169 (94.9)	9 (5.1)	0	

(continued)

Table (continued).

Study (Year)	Disease Group	Genotype Frequency			Ethnic Group
		GG	AG	AA	
Carvalho et al (2009)[25]	Posttransplant viral infection	83 (95.4)	4 (4.6)	0	Italian
	No viral infection	127 (94.8)	7 (5.2)	0	
Mutlubas et al (2009)[28]	All transplant recipients	10 (14.5)	15 (21.7)	44 (63.8)	Turkish
	CAN+	3 (15.8)	3 (15.8)	13 (68.4)	
	CAN–	7 (35.0)	12 (60.0)	1 (5.0)	
	Control	–	7 (6.1)	108 (93.9)	
Woehrle et al (2008)[23]	Septic shock	308 (94.8)	17 (5.2)	0	German
Moura et al (2008)[24]	*Helicobacter pylori*	252 (98.4)	4 (1.6)	0	Brazilian
	H pylori+/gastritis	156 (99.4)	1 (0.6)	0	
	H pylori+/duodenal ulcer	69 (98.6)	1 (1.4)	0	
Present study	Superficial bladder cancer	112 (100.0)	0	0	Tunisian

Arg677Trp (A → T) = arginine to tryptophan substitution at position 677 polymorphism of TLR2; Arg753Gln (A → G) = arginine to glutamine substitution at position 753 polymorphism of TLR2; CAN = chronic allograft nephropathy.

relative importance of these polymorphisms could not be definitely determined. However, 67 of 112 individuals (59.8%) without these polymorphisms responded to BCG in the present study.

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REFERENCES

1. Takeda K, Akira S. Toll-like receptors in innate immunity. *Int Immunol*. 2005;17:1–14.
2. Beutler B, Jiang Z, Georgel P, et al. Genetic analysis of host resistance: Toll-like receptor signaling and immunity at large. *Annu Rev Immunol*. 2006;24:353–389.
3. Bafica A, Scanga CA, Feng CG, et al. TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to Mycobacterium tuberculosis. *J Exp Med*. 2005;202:1715–1724.
4. Akaza H, Hinotsu S, Aso Y, et al, for the Bladder Cancer BCG Study Group. Bacillus Calmette-Guérin treatment of existing papillary bladder cancer and carcinoma in situ of the bladder. Four-year results. *Cancer*. 1995;75:552–559.
5. Decobert M, Larue H, Bergeron A, et al. Polymorphisms of the human NRAMP1 gene are associated with response to bacillus Calmette-Guerin immunotherapy for superficial bladder cancer. *J Urol*. 2006;175:1506–1511.
6. Basturk B, Yavascaoglu I, Oral B, et al. Cytokine gene polymorphisms can alter the effect of Bacillus Calmette-Guérin (BCG) immunotherapy. *Cytokine*. 2006;35:1–5.
7. Tsuji S, Matsumoto M, Takeuchi O, et al. Maturation of human dendritic cells by cell wall skeleton of Mycobacterium bovis bacillus Calmette-Guérin: Involvement of toll-like receptors. *Infect Immun*. 2000;68:6883–6890.
8. Akaza H. New strategy of bio-chemoprevention on recurrence of superficial bladder cancer based on a hypothesis of the mechanism of recurrence. *Gan To Kagaku Ryoho*.1997;24 (Suppl 1):253–256.
9. Bochud PY, Hawn TR, Aderem A. Cutting edge: A toll-like receptor 2 polymorphism that is associated with lepromatous leprosy is unable to mediate mycobacterial signaling. *J Immunol*. 2003;170:3451–3454.
10. Bochud PY, Hawn TR, Siddiqui MR, et al. Toll-like receptor 2 (TLR2) polymorphisms are associated with reversal reaction in leprosy. *J Infect Dis*. 2008;197:253–261.
11. Kang TJ, Chae GT. Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. *FEMS Immunol Med Microbiol*. 2001;31:53–58.
12. Ben-Ali M, Barbouche MR, Bousnina S, et al. Toll-like receptor 2 Arg677Trp polymorphism is associated with susceptibility to tuberculosis in Tunisian patients. *Clin Diagn Lab Immunol*. 2004;11:625–626.
13. Ogus AC, Yoldas B, Ozdemir T, et al. The Arg753Gln polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. *Eur Respir J*. 2004;23:219–223.
14. de Reijke TM, Vos PC, de Boer EC, et al. Cytokine production by the human bladder carcinoma cell line T24 in the presence of bacillus Calmette-Guerin (BCG). *Urol Res*. 1993;21: 349–352.

15. Miyazaki J, Kawai K, Oikawa T, et al. Uroepithelial cells can directly respond to Mycobacterium bovis bacillus Calmette-Guérin through Toll-like receptor signalling. *BJU Int*. 2006;97:860–864.
16. Chen F, Zhang G, Iwamoto Y, See WA. Bacillus Calmette-Guerin initiates intracellular signaling in a transitional carcinoma cell line by cross-linking alpha 5 beta 1 integrin. *J Urol*. 2003;170: 605–610.
17. Sobin DH, Wittekind CH, eds. *TNM Classification of Malignant Tumours*. 6th ed. New York, NY: Wiley-Liss; 2002:199–202.
18. Epstein JI, Amin MB, Reuter UR, Mostofi FK, for the Bladder Consensus Conference Committee. The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. *Am J Surg Pathol*. 1998;22:1435–1448.
19. Kang TJ, Lee SB, Chae GT. A polymorphism in toll-like receptor 2 is associated with IL-12 production from monocyte in lepromatous leprosy. *Cytokine.* 2002;20:56–62.
20. Malhotra D, Relhan V, Reddy BS, Bamezai R. TLR2 Arg677Trp polymorphism in leprosy: Revisited. *Hum Genet*. 2005;116:413–415.
21. Schröder NW, Hermann C, Hamann L, et al. High frequency of polymorphism Arg753Gln of the Toll-like receptor-2 gene detected by a novel allele-specific PCR. *J Mol Med*. 2003;81:368–372.
22. Texereau J, Chiche JD, Taylor W, et al. The importance of Toll-like receptor 2 polymorphisms in severe infections. *Clin Infect Dis*. 2005;41(Suppl 7):S408–S415.
23. Woehrle T, Du W, Goetz A, et al. Pathogen specific cytokine release reveals an effect of TLR2 Arg753Gln during Candida sepsis in humans. *Cytokine*. 2008;41:322–329.
24. Moura SB, Almeida LR, Guerra JB, et al. Toll-like receptor (TLR2, TLR4 and TLR5) gene polymorphisms and Helicobacter pylori infection in children with and without duodenal ulcer. *Microbes Infect*. 2008;10:1477–1483.
25. Carvalho A, Cunha C, Carotti A, et al. Polymorphisms in Toll-like receptor genes and susceptibility to infections in allogeneic stem cell transplantation. *Exp Hematol*. 2009;37:1022–1029.
26. Moens L, Verhaegen J, Pierik M, et al. Toll-like receptor 2 and Toll-like receptor 4 polymorphisms in invasive pneumococcal disease. *Microbes Infect*. 2007;9:15–20.
27. Lorenz E, Mira JP, Cornish KL, et al. A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. *Infect Immun*. 2000;68:6398–6401.
28. Mutlubas F, Mir S, Berdeli A, et al. Association between Toll-like receptors 4 and 2 gene polymorphisms with chronic allograft nephropathy in Turkish children. *Transplant Proc*. 2009;41: 1589–1593.
29. Park Y, Park S, Yoo E, et al. Association of the polymorphism for Toll-like receptor 2 with type 1 diabetes susceptibility. *Ann N Y Acad Sci*. 2004;1037:170–174.
30. Chen PL, Eng HL, Chou MH, et al. Genetic polymorphisms of viral infection-associated Toll-like receptors in Chinese population. *Transl Res*. 2007;150:311–318.
31. Ryu YJ, Kim EJ, Koh WJ, et al. Toll-like receptor 2 polymorphisms and nontuberculous mycobacterial lung diseases. *Clin Vaccine Immunol*. 2006;13:818–819.

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